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SUBMITTED: 2001-12-21 15:28:59
PRINTED: 2001-12-26 10:39:32
REQUEST NO.: NIH-10096740
SENT VIA: LOAN DOC
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NIH Fiche to Paper Journal

TITLE:	BAILLIERE'S CLINICAL ENDOCRINOLOGY AND METABOLISM
PUBLISHER/PLACE:	Bailliere Tindall London
VOLUME/ISSUE/PAGES:	1996 Jan;10(1):177-87 177-87
DATE:	1996
AUTHOR OF ARTICLE:	Milligan G
TITLE OF ARTICLE:	Endocrine disorders associated with mutations in g
ISSN:	0950-351X
OTHER NOS/LETTERS:	Library reports holding volume or year 8704785 8734456
SOURCE:	PubMed
CALL NUMBER:	W1 BA46EE
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Endocrine disorders associated with mutations in guanine nucleotide binding proteins

GRAEME MILLIGAN

Heterotrimeric guanine nucleotide binding proteins (G-proteins) play central roles in cellular information processing by allowing communication between heptahelical G-protein-coupled receptors and both enzymes which control the rate of production of intracellular second messengers and various classes of ion channels (Birnbaumer et al, 1990; Kaziro et al, 1991). The cellular level of cyclic adenosine monophosphate (cAMP) plays a key role in determining the growth rate of many cells (in either a positive or negative manner depending on the particular cell type) (Dumont et al, 1989; Burgering and Bos, 1995). As such, dysregulation of the control of cAMP production or degradation might be anticipated to result in changes in cellular growth control. Regulation of the activation of isoforms of protein kinase C has also been implicated in the regulation of cellular growth control. The activation status of certain members of this class of kinase is controlled by the production of *sn* 1-2 diacylglycerol derived via G-protein-linked receptor-catalysed hydrolysis of certain membrane phospholipids. Thus, a second endocrine-regulated G-protein-coupled pathway may contribute to cellular growth and differentiation (Seuwen and Pouyssegur, 1992). An analysis of human diseases, which result from mutations in individual G-proteins leading to dysregulation of these pathways, will form the basis of this chapter.

G-proteins consist of three non-identical, non-covalently associated subunits, α, β and γ. Approximately 20 distinct human G-protein α subunits have now been identified. These are mainly the products of individual genes but in the cases of G_s (the G-protein responsible for receptor-mediated stimulation of adenylyl cyclase and thus of cAMP levels) and of G_o (a G-protein largely restricted to neural and endocrine tissues which functions to regulate the opening of voltage-operated Ca^{2+} channels) splice variation further increases the diversity. The $\beta\gamma$ subunits exist physiologically as a non-dissociating complex and thus can be viewed as a single entity. These subunits are also present in multiple forms, with currently five β and seven γ subunit cDNA species having been identified. In the unactivated state the G-proteins exist as heterotrimeric complexes with the nucleotide binding pocket of the α subunit being occupied by guanosine diphosphate (GDP). Upon hormone

Baillière's Clinical Endocrinology and Metabolism—
Vol. 10, No. 1, January 1996
ISBN 0–7020–2072–9

occupation of a relevant receptor the rate of release of GDP from this site is increased markedly and subsequently it is replaced by guanosine triphosphate (GTP) (Bourne et al, 1990). The activated G-protein is then believed to physically dissociate into a GTP-liganded α subunit and the $\beta\gamma$ complex. Each of these complexes has the potential to regulate the activity of specific isoforms of effector enzymes. Inactivation of the GTP-bound G-protein is dependent upon the intrinsic GTPase activity of the α subunit. This acts as a timer to limit temporally the active state and thus to prevent constitutive activation.

The activity of adenylyl cyclase is regulated directly by two sets of G-proteins. Stimulatory signals are transduced via the splice variants of $G_s\alpha$ and inhibitory signals via the G_i-like G-proteins. Three related G-protein α subunits, G_i1, G_i2 and G_i3 can contribute to this in various situations but $G_i2\alpha$ is generally regarded as the major inhibitory regulator (Simonds et al, 1989; McKenzie and Milligan, 1990).

The first indication that alterations in the structure of G-protein α subunits could alter their activity and lead to the development of disease was the realization that the exotoxin of *Vibrio cholerae* possessed an adenosine diphosphate (ADP)-ribosyltransferase activity and that the α subunit of G_s was the key cellular substrate for this activity. Ingestion of the cholera bacterium leads to sustained activation of adenylyl cyclase activity in cells of the intestinal epithelium, elevated levels of cAMP and subsequently transport of Cl^- ions and water from the cells. The effect of cholera toxin-catalysed ADP-ribosylation of $G_s\alpha$ is to slow and indeed virtually eliminate the GTPase activity of the α subunit (Bourne et al, 1990): therefore the G-protein becomes essentially constitutively active, resulting in maintained and hormone-independent activation of adenylyl cyclase. The target amino acid for cholera toxin-catalysed ADP-ribosylation of $G_s\alpha$ is Arg201 and designed mutations at this position also result in constitutive activation of the G-protein (Freissmuth and Gilman, 1989). As this arginine is a key component of the regulatory turn-off mechanism of this G-protein it is not surprising that other G-protein α subunits have an arginine residue at an equivalent position in their primary sequence. Although cholera toxin cannot modify this arginine in the majority of other G-proteins, designed mutations have shown that alterations in this position also result in constitutive activation. Such mutations have been enlightening in determining the downstream effector polypeptides which are regulated by individual G-proteins. A combination of these observations led to the concept that such mutations in these G-proteins might be responsible for, or contribute to, a variety of human endocrine disorders in which the normal regulation of cAMP production was abrogated.

G-PROTEIN MUTATIONS IN AUTONOMOUS ENDOCRINE TUMOURS

Constitutive activation of adenylyl cyclase in the pituitary can result in cellular hyperplasia. The overproduction of pituitary-derived hormones

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was demonstrated by targeted transgenic expression of the enzymatically active subunit of cholera toxin in mice. This resulted in pituitary hyperplasia (presumably from the elevated cAMP levels) and gigantism (presumably via over-production of growth hormone) (Burton et al, 1991). Identification of a series of patients with growth hormone-secreting pituitary adenomas in which high basal adenylyl cyclase activity and poor responsiveness of the adenylyl cyclase to stimulatory agents such as growth hormone-releasing hormone (Vallar et al, 1987) suggested constitutive activation of the adenylyl cyclase cascade in cells of these tumours. Analysis of DNA derived from these tumours demonstrated that a considerable number of them harboured an activating mutation of $G_s\alpha$ (Landis et al, 1989). These were clearly the result of somatic mutation as the mutant allele was not observed in genomic DNA isolated from peripheral blood cells, which contained only the wild-type sequence. Mutational alterations were found at two distinct codons: Arg201 and Gln227. As noted above, Arg201 is the site for cholera toxin-catalysed ADP-ribosylation. Gln227 is in a section of the primary sequence which comprises part of the guanine nucleotide binding pocket of the G-protein and is in an equivalent position to Gln61 in the small molecular mass G-protein p21*ras*. Mutation at this position in p21*ras* has been observed to result in activation of the protein and subsequent tumourigenesis in cells expressing such an allele (Bos, 1989). Subsequent studies have confirmed the presence of such activating mutations of $G_s\alpha$ (often designated *gsp* mutations because of their oncogenic potential and the tradition of providing protein products of oncogenes with a simple three letter descriptor) in tumours from such patients (Clementi et al, 1990; Klibanski, 1990; Landis et al, 1990; Lyons et al, 1990; Spada et al, 1990; Drews et al, 1992; Adams et al, 1993; Yoshimoto et al, 1993; Tordjman et al, 1993).

These activating mutations of $G_s\alpha$, as might be anticipated, appear only to be associated with pituitary adenomas which display constitutive activity of adenylyl cyclase and not in those with normal regulation of this signalling cascade (Landis et al, 1989; Lyons et al, 1990). As the thyroid is also a tissue in which elevated cAMP levels is a growth stimulatory signal, equivalent mutations have been sought and identified in some autonomously functioning thyroid adenomas (Lyons et al, 1990; O'Sullivan et al, 1991) and a limited number of thyroid carcinomas (Suarez et al, 1991). No reports of activating mutations of $G_s\alpha$ associated with melanomas or tumours of the adrenal cortex have appeared, even though these tissues contain cell types in which cAMP is a positive growth stimulus. To date, the only other equivalent mutations reported for a G-protein α subunit associated with human disease have been Arg179 (the position equivalent to Arg201 in $G_s\alpha$) mutations in $G_i2\alpha$ in small numbers of adrenal medulla ovarian tumours (Lyons et al, 1990). This association has led to the mutant $G_i2\alpha$ proteins being named *gip2* to indicate its oncogenic potential in a similar manner to the use of *gsp* for the activating mutations of $G_s\alpha$.

Although not a mutant protein, Selzer et al (1993) have noted that the expression of $G_i1\alpha$ is tightly regulated in vivo and in primary cultures of

thyroid epithelial cells by thyrotrophin. This regulation is lost in autonomous adenomas of the thyroid where G_i1 is expressed independently of the presence of thyrotrophin. The contribution of this G-protein to the development of the condition, however, is not currently clear.

ALBRIGHT HEREDITARY OSTEODYSTROPHY

Albright hereditary osteodystrophy (AHO) is a disorder inherited as an autosomal dominant and is identified clinically by short stature, obesity, subcutaneous ossifications, focal skeletal defects and rounded facies. When associated with resistance to a range of hormones which function via $G_s\alpha$ to raise intracellular concentrations of cAMP (e.g. parathyroid hormone, luteinizing hormone, glucagon) it is termed pseudohypoparathyroidism type Ia (PHP 1a). The observation of AHO without PHP1a in relatives of these patients is described as pseudopseudohypoparathyroidism (PPHP). Membranes from a variety of tissues of many AHO patients show a reduction of approximately 50% compared to control, in functional $G_s\alpha$ activity as measured by reconstitution of adenylyl cyclase activity to membranes of S49 cyc^- cells (which genetically lack $G_s\alpha$) by detergent extracts of membranes from the tissues of the patients. Levels of each of $G_s\alpha$ protein, measured by immunoblotting (Patten and Levine, 1990), and $G_s\alpha$ mRNA, as detected by Northern blots (Carter et al, 1987; Levine et al, 1988), are also substantially reduced (although levels of this mRNA are not reduced in all kindreds). Reduced levels of $G_s\alpha$ activity have also been noted in PPHP patients (Levine et al, 1986). It is thus unclear what other alterations must be manifest to result in the clinical phenotype of AHO with PHP1a. Speculation has centred on other elements of the cellular cAMP generation and degradation machinery such as cAMP phosphodiesterases (Spiegel, 1990), but no data to support such a contention are currently available.

By contrast, patients with isolated resistance to parathyroid hormone in the absence of AHO (termed pseudohypoparathyroidism type 1b), have normal immunologically detectable cellular levels of $G_s\alpha$ (Patten and Levine, 1990). Such patients may have defects in the parathyroid hormone receptor (Silve et al, 1986). Clearly, a variety of genetic alterations could be responsible for the reduced tissue levels and activity of $G_s\alpha$ associated with AHO and the differences between kindreds in levels of relevant mRNA. Genetic analysis has indeed proved this to be the case (Patten et al, 1990; Weinstein et al, 1990; Lin et al, 1992; Weinstein et al, 1992; Miric et al, 1993; Schwindinger et al, 1994; see Miric and Levine, 1992 for review). The first mutation noted in the $G_s\alpha$ gene associated with some AHO patients was a single base substitution in one allele resulting in the conversion of the initiator codon ATG (methionine) to GTG (valine) (Patten et al, 1990). The normal 45 kDa $G_s\alpha$ in membranes of erythrocytes of these patients was substantially reduced compared to controls. However, an apparent 77 kDa polypeptide containing immunological information consistent with the presence of a C-terminal $G_s\alpha$ epitope but not an N-terminal region was observed. While one hypothesis would have anticipated the

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appearance of a truncated form of the G-protein resulting from initiation from an internal, in frame, AUG codon, no shorter species corresponding immunologically to $G_s\alpha$ were observed (Patten et al, 1990). The nature of the 77 kDa polypeptide, however, remains to be fully explored. A considerable range of other mutations in individual AHO kindreds have now been shown. These include a G to C substitution at the donor splice junction of intron 10 of the $G_s\alpha$ gene (Weinstein et al, 1990), which would be anticipated to result in abnormal RNA splicing; a coding frameshift created by a single base deletion within exon 10 (Weinstein et al, 1990) and a single amino acid mutation (R385H) (Schwindinger et al, 1994) close to the C-terminus of $G_s\alpha$. This mutation is in a region known to be involved in contacts between receptors and $G_s\alpha$ as β2-adrenoceptor contact with $G_s\alpha$ is abolished in lymphoma S49 *unc* cells which harbour a R389P mutation in $G_s\alpha$ (Sullivan et al, 1987).

McCUNE–ALBRIGHT SYNDROME

The McCune–Albright syndrome is an interesting example of mosaicism. This mechanism was first suggested by Happle (1986) in view of the multisite tissue abnormalities and the observed patterns of hyperpigmentation in this disorder. The condition is defined classically by the presence of cutaneous hyperpigmentation, polyostotic fibrous dysplasia and a range of endocrine hyperfunctions of varying degrees in systems anticipated to be coupled to the stimulation of adenylyl cyclase. As the endocrinopathies may include sexual precocity and autonomous adrenal hyperplasia, and the unusual 'cafe au lait' pigmentation arises from excess functioning of melanocytes, then it was reasonable to suggest that the condition might result from over-activity of the adenylyl cyclase cascade. Furthermore, the bone lesions associated with the disorder resemble those that occur in primary hyperparathyroidism. As with the examples of pituitary and thyroid adenomas discussed above, patients with McCune–Albright syndrome harbour activating mutations (*gsp*) of the $G_s\alpha$ gene. Weinstein and Shenker (1993) and Weinstein et al (1991) have reported the identification of Arg201 mutations of $G_s\alpha$ in tissues from 15 McCune–Albright syndrome patients and others have reported equivalent mutations (Schwindinger et al, 1992). DNA extracted directly from a 'cafe au lait' patch of skin has also been shown to contain the activating $G_s\alpha$ mutation (Schwindinger et al, 1992). As the presence of the mutated allele could not be shown in all tissues, and to variable extents in tissues displaying its presence, such data support the notion of mosaicism.

The mosaicism in McCune–Albright syndrome has been suggested to occur as the result of a post-zygotic somatic cell mutation (Happle, 1986). This would account appropriately for the lack of genetic inheritance of the condition (perhaps due to lethality of an equivalent germ line mutation) and the variability in degree and distribution of the endocrine abnormalities in affected individuals. Furthermore, as cAMP is growth inhibitory in a wide range of tissues, malignancy is uncommon in the condition.

TESTITOXICOSIS

Leydig cells of the testis are stimulated to produce the androgen testosterone by the action of luteinizing hormone (LH). LH, produced by the anterior pituitary, binds to and activates a specific heptahelical G-protein-coupled receptor leading to the activation of $G_s\alpha$ and adenylyl cyclase and thus elevation of intracellular cAMP levels. Potential activating mutations in the LH receptor pathway might be anticipated to lead to constitutive production of testosterone and thus to the development of cases of testitoxicosis or familial male precocious puberty. As with other G-protein-linked signalling cascades, such alterations could potentially be produced by mutation of receptor, G-protein or adenylyl cyclase. Strong linkage of a single base alteration (A→G), resulting in substitution of Gly 578 in the putative sixth transmembrane helix by aspartate, has been reported in a study of eight different families with gonadotrophin-independent generation of testosterone and Leydig cell hyperplasia in the presence of prepubertal levels of circulating LH (Shenker et al, 1993). Transient expression in COS cells of an LH receptor cDNA mutated in this position resulted in elevated production of cAMP in the absence of agonist (Skenker et al, 1993), demonstrating this mutation to result in constitutive activation of the adenylyl cyclase cascade. Clearly, an activating mutation of $G_s\alpha$ similar to those observed in pituitary adenomas (see above) would be expected to result in a similar phenotype.

Examination of two unrelated boys with an apparently paradoxical mixture of testotoxicosis and pseudohypothyroidism type 1a (Nakomoto et al, 1993) led to the detection of a novel activating mutant of $G_s\alpha$ with a number of intruiging features which are seemingly able to account for this unexpected combination of features (Iiri et al, 1994). In these patients a mutation in the $G_s\alpha$ gene resulted in the replacement of Ala 366 by serine (A366S) (Iiri et al, 1994). To examine how such a mutation in $G_s\alpha$ could result in an apparent gain of G-protein function in the testis but be consistent with the apparent reduction of function in other tissues, an A366S mutant $G_s\alpha$ cDNA was constructed (incorporating an epitope tag for easy immunological detection) and compared to the epitope-tagged wild-type G-protein in a range of assays. The purified mutant protein was shown to bind the poorly hydrolysed analogue of GTP, [^{35}S]GTPγS, much more rapidly than the wild-type protein. However, with prolonged incubation the maximal level of binding was not different (Iiri et al, 1994). This difference reflected a markedly elevated rate of release of bound GDP (which is normally the rate-limiting step for guanine nucleotide exchange) by the A366S mutant. As such, in the cells of the patient it would be anticipated that the mutant protein would spend a greater fraction of its time in the GTP-bound and hence activated state leading to constitutive activation of adenylyl cyclase. However, while consistent with the clinical gain of function features of testitoxicosis this is apparently not consistent with the anticipated loss of function features of pseudohypothyroidism type 1a. When expressed in cultured cells grown at 37°C the A366S mutant $G_s\alpha$ protein was shown to be much less stable than the wild-type protein, thus leading to its rapid degradation.

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Such differential rates of degradation were not observed at 33°C. It is thus likely that in the patients, at the temperature of the testis, the mutant protein was relatively stable and thus could function as a mutant capable of producing constitutive activation of adenylyl cyclase. It may be that steady-state levels of the mutant protein in other tissues of the body are low due to the short half-life of the protein, such that the level of expressed protein, while still constitutively active, is insufficient to produce even a normal level of adenylyl cyclase activation. However, it should be noted in the cellular transfection experiments reported by Iiri et al (1994) that the levels of the A336S $G_s\alpha$ mutant achieved following transfection, whilst lower than that of wild-type protein, was sufficient to cause a greater elevation of cAMP levels than that produced by the wild-type protein. It thus requires further analysis of levels and function of the mutant $G_s\alpha$ protein in such patients to confirm that the reported clinical phenotype can be explained solely on the basis of this single mutation.

LACK OF MUTATIONS IN OTHER G-PROTEINS ASSOCIATED WITH ENDOCRINE DISORDERS

To date there have been no reports of gain or loss of function in endocrine disorders resulting from mutations in members of families of heterotrimeric G-protein α subunits apart from $G_s\alpha$ and $G_i2\alpha$ (and, as noted above, those associated with a $G_i2\alpha$ mutation are extremely limited). At first sight, this is a surprise as the generation and expression of constitutively activating mutant cDNA species of members of the G_q/G_{11} and G_{12} families of G-proteins (which are widely expressed) have been reported to result in alterations of growth control (both positive and negative) and in some cases in transformation of fibroblast cell lines. For example, it has been reported that the GTPase deficient mutation of $G_q\alpha$ ($G_q\alpha$ Q209L) can cause transformation of NIH 3T3 cells (Kalinec et al, 1992; De Vivo et al, 1992). However, such observations are not universal: Wu et al (1992) found this mutant to be growth inhibitory to NIH 3T3 cells. Expression of many of these mutants results in a strong agonist-independent activation of phospholipase Cβ activity and certainly many G-protein-linked receptors for mitogens activate members of the G_q-family of G-proteins (Wu et al, 1992; Qian et al, 1994) leading to stimulation of phospholipase Cβ activity. Such results clearly indicate that activation of phospholipase Cβ is an insufficient indicator of mitogenic potential. Many G-protein-linked mitogenic ligands which do activate phospholipase Cβ also regulate a variety of other enzyme cascades including phospholipase A2 and the MAP kinases. A number of receptors for other G-protein-linked mitogenic ligands interact with $G_i2\alpha$ (van Corven et al, 1993; Winitz et al, 1993; Alblas et al, 1993). These may produce their regulation of mitogenesis either by provision of G-protein βγ subunits (Crespo et al, 1994; Faure et al, 1994; Koch et al, 1994), which directly or indirectly lead to activation of MAP kinases, or by reduction of cellular cAMP levels (Sevetson et al, 1993; Hordijk et al, 1994). Mutational activation of $G_{12}\alpha$ has also been reported

to be highly oncogenic when expressed in NIH 3T3 cells (Xu et al, 1993; Jiang et al, 1993). The lack of identified mutations in human disease in these other G-proteins equivalent to the *gsp* and *gip2* mutations discussed above is thus somewhat surprising and the reasons for this remain to be clearly elucidated.

SUMMARY

The basis for a number of relatively rare endocrine diseases, which present clinically with features of AHO, have been shown conclusively to result from mutations in the $G_s\alpha$ gene which interfere with the expression of functional protein. Individual kindreds display a range of specific mutations in this gene. A further series of disorders result from somatic mutations of the $G_s\alpha$ gene which result in constitutive activation (in one case probably with a concomitant decrease in stability of the expressed protein). When such a mutation occurs in early embryogenesis it can result in a pattern of mosaicism of expression of clinical features in the patient. Despite these cases, equivalent alterations in other G-protein α subunit genes seem to be of limited importance in human disease. This is despite biochemical data from a range of experimental cell models which indicate that such mutations can have potent effects on cell growth and division.

REFERENCES

Adams EF, Brockmeier S, Friedman E et al (1993) Clinical and biochemical characteristics of acromegalic patients harboring *gsp*-positive and *gsp*-negative pituitary tumors. *Neurosurgery* **33:** 198–203.

Alblas J, van Corven EJ, Hordijk PL et al (1993) G_i-mediated activation of the $p21^{ras}$-mitogen-activated protein kinase pathway by α_2-adrenergic receptors expressed in fibroblasts. *Journal of Biological Chemistry* **268:** 22 235–22 238.

Birnbaumer L, Abramowitz J & Brown AM (1990) Receptor-effector coupling by G proteins. *Biochemica et Biophysica Acta* **1031:** 163–224.

Bos JL (1989) *Ras* oncogenes in human cancer: a review. *Cancer Research* **49:** 4682–4689.

Bourne HR, Sanders DA & McCormick F (1990) The GTPase superfamily: A conserved switch for diverse cell functions. *Nature (London)* **348:** 125–132.

Burgering BMT & Bos JL (1995) Regulation of *ras*-mediated signalling: more than one way to skin a cat. *Trends in Biochemical Sciences* **20:** 18–22.

Burton FH, Hasel KW, Bloom FE & Sutcliffe JG (1991) Pituitary hyperplasia and gigantism in mice caused by a cholera toxin transgene. *Nature (London)* **350:** 74–77.

Carter A, Bardin C, Collins R et al (1987) Reduced expression of multiple forms of the α subunit of the stimulatory GTP-binding protein pseudohypothyroidism type 1a. *Proceedings of the National Academy of Sciences of the USA* **84:** 7266–7269.

Clementi E, Malgaretti N, Meldolesi J & Taramelli R (1990) A new constitutively activating mutation of the G_s protein α subunit-*gsp* oncogene is found in human pituitary tumours. *Oncogene* **5:** 1059–1061.

Crespo P, Xu N, Simonds WF & Gutkind JS (1994) *Ras*-dependent activation of MAP kinase pathway mediated by G-protein βγ subunits. *Nature (London)* **369:** 418–420.

De Vivo M, Chen J, Codina J & Iyengar R (1992) Enhanced phospholipase C stimulation and transformation in NIH-3T3 cells expressing Q209L G_q-α–subunits. *Journal of Biological Chemistry* **267:** 18 263–18 266.

Drews RT, Gravel RA & Collu R (1992) Identification of G protein α subunit mutations in human

d in NIH 3T3 cells (Xu et al, 1993;
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3) Clinical and biochemical characteristics of *gsp*-negative pituitary tumors. *Neurosurgery* **33:**

G_i-mediated activation of the $p21^{ras}$-mitogen-
ic receptors expressed in fibroblasts. *Journal of*

0) Receptor-effector coupling by G proteins.

iew. *Cancer Research* **49:** 4682–4689.
e GTPase superfamily: A conserved switch for
–132.
nediated signalling: more than one way to skin

91) Pituitary hyperplasia and gigantism in mice
don) **350:** 74–77.
xpression of multiple forms of the α subunit of
ypothyroidism type 1a. *Proceedings of the*
66–7269.
1990) A new constitutively activating mutation
nd in human pituitary tumours. *Oncogene* **5:**

Ras-dependent activation of MAP kinase path-
London) **369:** 418–420.
nanced phospholipase C stimulation and trans-
$_q$-α–subunits. *Journal of Biological Chemistry*

n of G protein α subunit mutations in human

growth hormone (GH) and GH/prolactin-secreting pituitary tumours by single-strand conformation polymorphism. *Molecular and Cellular Endocrinology* **87:** 125–129.

Dumont JE, Jauniaux J-C & Roger PP (1989) The role of cyclic AMP-mediated stimulation of cell proliferation. *Trends in Biochemical Sciences* **14:** 67–71.

Faure M, Voyno-Yasenetskaya TA & Bourne HR (1994) cAMP and βγ subunits of heterotrimeric G proteins stimulate the mitogen-activated protein kinase pathway in COS-7 cells. *Journal of Biological Chemistry* **269:** 7851–7854.

Freissmuth M & Gilman AG (1989) Mutations of $G_s\alpha$ designed to alter the reactivity of the protein with bacterial toxins. Substitutions at Arg187 result in a loss of GTPase activity. *Journal of Biological Chemistry* **264:** 21 907–21 964.

Happle R (1986) The McCune–Albright syndrome: A lethal gene surviving by mosaicism. *Clinical Genetics* **29:** 321–324.

Hordijk PL, Verlaan I, Jalink K et al (1994) cAMP abrogates the $p21^{ras}$-mitogen-activated protein kinase pathway in fibroblasts. *Journal of Biological Chemistry* **269:** 3534–3538.

Iiri T, Herzmark P, Nakamoto JM et al (1994) Rapid GDP release from $G_s\alpha$ in patients with gain and loss of endocrine function. *Nature (London)* **371:** 164–168.

Jiang H, Wu D & Simon, MI (1993) The transforming activity of activated Gα12. *FEBS Letters* **330:** 319–322.

Kalinec G, Nazarali AJ, Hermouet et al (1992) Mutated α subunit of the G_q protein induces malignant transformation in NIH 3T3 cells. *Molecular and Cellular Biology* **12:** 4687–4693.

Kaziro Y, Itoh H, Kozasa T et al (1991) Structure and function of signal-transducing GTP-binding proteins. *Annual Review of Biochemistry* **60:** 349–400.

Klibanski A (1990) Editorial: Further evidence for a somatic mutation theory in the pathogenesis of human pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* **71:** 1415A–1415C.

Koch WJ, Hawes BE, Allen LF & Lefkowitz RJ (1994) Direct evidence that G_i-coupled receptor stimulation of mitogen-activated protein kinase is mediated by $G_{\beta\gamma}$ activation of $p21^{ras}$ *Proceedings of the National Academy of Sciences of the USA* **91:** 12706–12710.

Landis CA, Masters SB, Spada A et al (1989) GTPase inhibiting mutations activate the α chain of G_s and stimulate adenylyl cyclase in human pituitary tumours. *Nature (London)* **340:** 692–696.

Landis CA, Harsh G, Lyons J et al (1990) Clinical characteristics of acromegalic patients whose pituitary tumors contain mutant G_s protein. *Journal of Clinical Endocrinology and Metabolism* **71:** 1416–1420.

Levine MA, Jap TS, Mauseth RS et al (1986) Activity of the stimulatory guanine nucleotide binding protein is reduced in erthyrocytes from patients with pseudohypoparathyroidism and pseudo-pseudohypoparathyroidism: Biochemical, endocrine, and genetic analysis of Albright's hereditary osteodystrophy in six kindreds. *Journal of Clinical Endocrinology and Metabolism* **62:** 497–502.

Levine MA, Ahn TG, Klupt SF et al (1988) Genetic deficiency of the α subunit of the guanine nucleotide-binding protein G_s as the molecular basis for Albright hereditary osteodystrophy. *Proceedings of the National Academy of Sciences of the USA* **85:** 617–621.

Lin CK, Hakakha MJ, Nakamoto JM et al (1992) Prevalence of three mutations in the $G_s\alpha$ gene among 24 families with pseudohypoparathyroidism type 1a. *Biochemical and Biophysical Research Communications* **189:** 343–349.

Lyons J, Landis CA, Harsh G et al (1990) Two G-protein oncogenes in human endocrine tumors. *Science* **249:** 655–659.

McKenzie FR & Milligan G (1990) δ-Opioid-receptor-mediated inhibition of adenylate cyclase is transduced specifically by the guanine-nucleotide-binding protein G_i2. *Biochemical Journal* **267:** 391–398.

Miric A & Levine MA (1992) Mutations within the gene encoding the stimulatory G-protein of adenylyl cyclase as the basis for Albright hereditary osteodystrophy. In Milligan G & Wakelam M (eds) *G-Proteins: Signal Transduction and Disease*, pp 29–46. London: Academic Press.

Miric A, Vechio JD & Levine MA (1993) Heterogenous mutations in the gene encoding the α subunit of the stimulatory G-protein of adenylyl cyclase in Albright hereditary osteodystrophy. *Journal of Clinical Endocrinology and Metabolism* **76:** 1560–1568.

Nakomoto JM, Jones EA, Zimmerman D et al (1993) A missense mutation in the $G_s\alpha$ gene is associated with pseudohypoparathyroidism type 1-A and gonadotropin-independent precocious puberty. *Clinical Research* **41:** 40A.

O'Sullivan C, Barton CM, Staddon SL et al (1991) Activating point mutations of the *gsp* oncogene in human thyroid adenomas. *Molecular Carcinogenesis* **4:** 345–349.

Patten JL & Levine MA (1990) Immunochemical analysis of the α subunit of the stimulatory G-protein of adenylyl cyclase in patients with Albright's hereditary osteodystrophy. *Journal of Clinical Endocrinology and Metabolism* **71:** 1208–2114.

Patten JL, Johns DR, Valle D et al (1990) Mutation in the gene encoding the stimulatory G-protein of adenylate cyclase in Albright's hereditary osteodystrophy. *New England Journal of Medicine* **322:** 1412–1419.

Qian, N-X, Russell M, Buhl AM & Johnson GJ (1994) Expression of GTPase-deficient $G_{\alpha}16$ inhibits Swiss 3T3 cell growth. *Journal of Biological Chemistry* **269:** 17 417–17 423.

Schwindinger WF, Francomano CA & Levine MA (1992) Identification of a mutation in the gene encoding the α subunit of the stimulatory G-protein of adenylyl cyclase in McCune–Albright syndrome. *Proceedings of the National Academy of Sciences of the USA* **89:** 5152–5156.

Schwindinger WF, Miric A, Zimmerman D & Levine MA (1994) A novel $G_s\alpha$ mutant in a patient with Albright hereditary osteodystrophy uncouples cell surface receptors from adenylyl cyclase. *Journal of Biological Chemistry* **269:** 25 387–25 391.

Selzer E, Wilfing A, Schiferer A et al (1993) Stimulation of human thyroid growth via the inhibitory guanine nucleotide binding (G) protein G_i: Constitutive expression of the G-protein α subunit $G_{i\alpha}$–1 in autonomous adenoma. *Proceedings of the National Academy of Sciences of the USA* **90:** 1609–1613.

Seuwen K & Pouyssegur J (1992) G-protein-controlled signal transduction pathways and the regulation of cell proliferation. *Advances in Cancer Research* **58:** 75–94.

Sevetson BR, Kong X & Lawrence Jr JC (1993) Increasing cAMP attenuates activation of mitogen-activated protein kinase. *Proceedings of the National Academy of Sciences of the USA* **90:** 10 305–10 309.

Silve C, Santora A, Breslau N et al (1986) Selective resistance to parathyroid hormone in cultured skin fibroblasts from patients with pseudohypoparathyroidism type 1b. *Journal of Clinical Endocrinology and Metabolism* **62:** 640–644.

Simonds WF, Goldsmith PK, Codina J et al (1989) G_{i2} mediates α_2-adrenergic inhibition of adenylyl cyclase in platelet membranes. In situ identification with Gα C-terminal antibodies. *Proceedings of the National Academy Sciences of the USA* **86:** 7809–7813.

Skenker A, Laue L, Kosugi S et al (1993) A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature (London)* **365:** 652–654.

Spada A, Arosio M, Bochicçhio D et al (1990) Clinical, biochemical, and morphological correlates in patients bearing growth hormone-secreting pituitary tumors with or without constitutively active adenylyl cyclase. *Journal of Clinical Endocrinology and Metabolism* **71:** 1421–1426.

Spiegel AM (1990) Albright's hereditary osteodystrophy and defective G-proteins. *New England Journal of Medicine* **322:** 1461–1462.

Suarez HG, du Villard JA, Caillou B et al (1991) *gsp* mutations in human thyroid tumours. *Oncogene* **6:** 677–679.

Sullivan KA, Miller RT, Masters SB et al (1987) Identification of receptor contact site involved in receptor-G-protein coupling. *Nature (London)* **330:** 758–760.

Tordjman K, Stern N, Ouaknine G et al (1993) Activating mutations of the $G_s\alpha$ gene in non-functioning pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* **77:** 765–769.

Vallar L, Spada A & Giannattasio G (1987) Altered G_s and adenylate cyclase activity in human GH-secreting pituitary adenomas. *Nature (London)* **330:** 566–568.

van Corven EJ, Hordijk PL, Medema RH et al (1993) Pertussis toxin-sensitive activation of $p21^{ras}$ by G-protein-coupled receptor agonists in fibroblasts. *Proceedings of the National Academy of Sciences of the USA* **90:** 1257–1261.

Weinstein LS & Shenker A (1993) G-protein mutations in human disease. *Clinical Chemistry* **26:** 333–338.

Weinstein LS, Gejman PV, Friedman E et al (1990) Mutations of the $G_s\alpha$ subunit gene in Albright hereditary osteodystrophy detected by denaturing gradient gel electrophoresis. *Proceedings of the National Academy of Sciences of the USA* **87:** 8287–8290.

Weinstein LS, Shenker A, Gejman PV et al (1991) Activating mutations of the stimulatory G-protein in the McCune–Albright syndrome. *New England Journal of Medicine* **325:** 1688–1695.

Weinstein LS, Gejman PV, de Mazancourt P et al (1992) A heterozygous 4-bp deletion mutation in the G_s alpha gene (GNAS1) in a patient with Albright hereditary osteodystrophy. *Genomics* **13:** 1319–1321.

Winitz S, Russell M, Qian N-X et al (1993) Involvement of ras and raf in the G_i-coupled acetylcholine

is of the α subunit of the stimulatory G-
t's hereditary osteodystrophy. *Journal of*
14.
ene encoding the stimulatory G-protein of
ophy. *New England Journal of Medicine*

ression of GTPase-deficient $G_{\alpha}16$ inhibits
ry **269:** 17 417–17 423.
) Identification of a mutation in the gene
of adenylyl cyclase in McCune–Albright
iences of the USA **89:** 5152–5156.
1994) A novel $G_s\alpha$ mutant in a patient with
surface receptors from adenylyl cyclase.

f human thyroid growth via the inhibitory
ve expression of the G-protein α subunit
ional Academy of Sciences of the USA **90:**

gnal transduction pathways and the regu-
rch **58:** 75–94.
g cAMP attenuates activation of mitogen-
cademy of Sciences of the USA **90:** 10 305–

ce to parathyroid hormone in cultured skin
roidism type 1b. *Journal of Clinical*

liates α_2-adrenergic inhibition of adenylyl
th Gα C-terminal antibodies. *Proceedings*
9–7813.
ly activating mutation of the luteinizing
Nature (London) **365:** 652–654.
chemical, and morphological correlates in
mors with or without constitutively active
nd Metabolism **71:** 1421–1426.
and defective G-proteins. *New England*

ons in human thyroid tumours. *Oncogene*

ation of receptor contact site involved in
8–760.
ing mutations of the $G_s\alpha$ gene in non-
ndocrinology and Metabolism **77:** 765–

adenylate cyclase activity in human GH-
56–568.
ssis toxin-sensitive activation of $p21^{ras}$ by
roceedings of the National Academy of

human disease. *Clinical Chemistry* **26:**

ions of the $G_s\alpha$ subunit gene in Albright
dient gel electrophoresis. *Proceedings of*
7–8290.
ng mutations of the stimulatory G-protein
rnal of Medicine **325:** 1688–1695.
eterozygous 4-bp deletion mutation in the
ereditary osteodystrophy. *Genomics* **13:**

as and raf in the G_i-coupled acetylcholine

muscarinic m2 receptor activation of mitogen-activated protein (MAP) kinase kinase and MAP kinase. *Journal of Biological Chemistry* **268:** 19 196–19 199.

Wu D, Lee CH, Rhee SG & Simon MI (1992) Activation of phospholipase C by the α subunits of the G_q and G_{11} proteins in transfected Cos-7 cells. *Journal of Biological Chemistry* **267:** 1811–1817.

Xu N, Bradley L, Ambdukar I & Gutkind JS (1993) A mutant α subunit of G_{12} potentiates the eicosanoid pathway and is highly oncogenic in NIH 3T3 cells. *Proceedings of the National Academy of Sciences of the USA* **90:** 6741–6745.

Yoshimoto K, Iwahana H, Fukada et al (1993) Rare mutations of the Gs alpha subunit gene in human endocrine tumors. *Cancer* **72:** 1386–1393.